REMARKS

Claims 1-23 are pending in this application. Claims 14-21 have been withdrawn from consideration. Claims 1-13 and 22-23 stand rejected. Reconsideration of this application in view of the following arguments is respectfully requested.

Declaration Under 37 C.F.R. §1.131

The Examiner states the §1.131 declaration is "adequate to overcome the Sallusto et al. reference, because neither the fax nor the declaration detail when the fax was sent." In an Amendment filed April 19, 1996, Applicants stated that why Sallusto et al. was not a proper and that a Declaration under 37 CFR §1.131 signed by Dr. Steinman removing Sallusto et al. as a reference would be filed. This Declaration (attached as Exhibit 1) was hand delivered to the USPTO on August 19, 1996. A copy of the self addressed postcard is attached as Exhibit 2. Applicants note that Sallusto et al. is not cited in the rejections under 35 U.S.C. §102 and 103, and therefore has been removed as a rejection. It is therefore believed Sallusto et al. has been overcome as a rejection.

Rejection of Claims 1-5 and 10 Under 35 U.S.C. 102(b)

Claims 1-5 and 10 stand rejected under 35 U.S.C. 102(b) as being anticipated by Markowicz et al. Specifically, the Examiner contends that Markowicz et al. teaches culturing dendritic cells in the presence of IL-4. Applicants traverse this ground of rejection.

The instantly claimed invention is directed to a method of producing a population of mature dendritic cells from proliferating dendritic cell precursors (See claim 1).

The method requires culturing a tissue source to produce proliferating dendritic cell precursors in the presence of an agent which inhibits the proliferation or maturation of nondendritic cell precursors. For a finding of anticipation each element of the claimed invention must be present in the cited reference. Markowicz et al. is clearly not such a reference. Markowicz et al. reports the effects of GM-CSF on the morphology and viability of dendritic cells isolated from human peripheral blood. However, Markowicz et al. does not teach or suggest that these cells proliferate in culture or disclose conditions for allowing dendritic cells to proliferate in culture. As Markowicz et al. does not disclose methods for culturing proliferating dendritic cell precursors or the addition of agents to specifically inhibit nondendritic cell precursors, the reference fails to anticipate the claimed invention.

In fact, Markowicz et al. specifically teaches away from applicants invention by teaching that GM-CSF does not cause proliferation of dendritic cells:

Specifically, Markowicz et al. states:

> As shown in Fig. 4. the number of differentiated (branched DC) increased as the concentration of GM-CSF in the culture increased. At any given concentration of the cytokine, however, the total number of viable cells as well as the number of branched cells per well remained stable over time suggesting that GM-CSF does not cause DC to divide and proliferate.

Markowicz et al., page 958, emphases added. Markowicz et al. therefore teaches away from concluding that the cultures have proliferating dendritic cell precursors. Since Markowicz et al. reports that dendritic cells comprise only a portion of the total cell population, even if there is proliferation in the culture which contributes to the maintenance of a stable cell number, there is no suggestion that such a proliferation of cells are dendritic cell precursors.

The data presented in Figure 4 indicates that the dendritic cells are about ten percent (10%) of the total cell population per well (10,000 - 20,000 total viable cells per culture period per well and about 1000 branched (dendritic) cells per well). Markowicz et al. states that:

> "Branched DC typically comprised 10-40% of the total number of viable cells in cultures supplemented with GM-CSF."

Markowicz et al., Page 959. Thus, if some dendritic cells are in fact dying, the number of dendritic cells may be maintained at a stable number by another form of non-proliferating cell maturing into a branched dendritic cell while other non-dendritic cells (e.g. macrophages or lymphocytes) proliferate keeping the total cell number constant. Markowicz also states that:

> "The remaining cells of the culture are primarily unbranched DC (DR+, Leu M3- cells capable of differentiating into branched adherent cells when transferred) and smaller numbers of macrophages and lymphoid cells."

Markowicz et al. Page 959. Consistent with this analysis is the lack of increase in dendritic cell number over time from day 11 to day 24 as shown in Figure 4 of Markowicz et al (page 959). If a population of dendritic cells were proliferating, as claimed by applicants, this number should increase over time. In contrast, applicants invention clearly demonstrates obtaining mature dendritic cells by the expansion of dendritic cell cultures from proliferating dendritic cell precursors (See Examples 7-9; pages 82-101).

In addition, Markowicz et al. reports, the proliferation of lymphocytes when IL-2 was added to dendritic cell cultures (page 959, right column). Markowicz et al.

therefore suggests other cell types which may also be candidates for maintaining a stable cell number if some cells are dying.

Applicants' invention is therefore clearly not anticipated by Markowicz et al. as Markowicz et al. does teach or suggest methods for producing mature dendritic cells from proliferating dendritic cell precursors or the addition of an agent to inhibit proliferation or maturation of nondendritic cell precursors.

In view of the above, Markowicz et al. does not anticipate applicants' claimed invention as it fails to demonstrate proliferating precursors of dendritic cells. Applicants respectfully request withdrawal of this ground of rejection.

Rejections of Claim 22 Under 35 U.S.C. §102 or Under 35 U.S.C. §103

The Examiner also asserts, that claim 22 is rejected under 35 U.S.C. §102(b) as anticipated by Markowicz et al or in the alternative that claim 22 is *prima facie* obvious over Markowicz et al. Applicants traverse this ground of rejection.

As stated above, Markowicz et al. does not teach or suggest that the reported dendritic cells proliferate in culture or that additional agents should be added that inhibit the maturation or proliferation of non-dendritic cell precursors. Therefore, Markowicz et al. does not teach or suggest the instantly claimed method which is directed towards methods of culturing and proliferating dendritic cells *in vitro* in the presence of factors that inhibits maturation of non-dendritic precursor cells to produce sufficient quantities of mature dendritic cells to be useful as adujants or for producing antigens. Absent such a teaching

Markowicz et al. can neither anticipate or render the instantly claimed invention obvious. Hence, withdrawal of the rejection and reconsideration are respectfully requested.

Rejection of Claims 6 and 11-12 Under 35 U.S.C. §103

Claims 6 and 11-12 stand rejected under 35 U.S.C. §103 because the Examiner contends they are unpatentable over Markowicz et al. as applied to claims 1-5 and 10 above and further in view of Jakoby et al.

Applicants traverse this rejection for the reason stated below.

The Examiner concedes that Markowicz et al. "differs from the claimed invention by not specifically indicating the exact concentration level of IL-4 and also by teaching the utilization of a slightly less concentration level of GM-CSF then that which is specifically claimed" (see page 4 of Office Action dated September 4, 1996). As argued above, Markowicz et al does not teach or suggest the production of mature dendritic cells from proliferating dendritic cell precursors or the addition of an agent to inhibit or prevent proliferation of non-dendritic cell precursors. Claims 6 and 11-12 (as they depend from claim 1) require proliferating dendritic cell precursors. Therefore there is no basis to rely on Markowicz et al. as a primary reference.

Jakoby et al. relates generally to generalized standards for tissue culture. Jakoby et al. does not discuss methods of culturing proliferating dendritic cells. Such disclosure is found only in applicants' disclosure. The Examiner's contention that cell culture readers may be "tailor[ed] to optimize experimental conditions" presumes there is a condition to be optimized. Since the condition is provided only by applicants' own

disclosure and not by Markowicz et al., the Examiner's rejection is without merit. Hence, the invention is not rendered obvious by Jakoby et al. either alone or in combination with Markowicz et al. Reconsideration and withdrawal of this rejection is respectfully requested.

Rejection of Claim 7 and 13 Under 35 U.S.C §103

Claim 7 and 13 are rejected under 35 U.S.C. §103 as being unpatentable over Markowicz et al. as applied to claims 1-5 and 10 above and further in view of Koch et al.

Applicants traverse this rejection for the reasons discussed below.

Koch et al. reports the use of TNF-α in cultures of murine epidermal Langerhans cells and not to the production of mature dendritic cells from proliferating cultures of dendritic cells. Furthermore, Koch does not teach or suggest the use of factors in the culture of proliferating dendritic cells which inhibit non-dendritic cell precursors. The Examiner concedes that Markowicz et al. does not teach that the "culture medium may further comprise TNF-alpha". Therefore, Koch et al. does not remedy the deficiency of Markowicz et al. as discussed above. Withdrawal of this rejection is respectfully requested.

Rejection of Claims 8-9 and 23 Under 35 U.S.C. §103

Claims 8-9 and 23 stand rejected under 35 U.S.C. §103 as being unpatentable over Markowicz et al. as applied to claims 1-5 and 10 above and further in view of Van Voorhis et al. or Ruley et al.

Applicants traverse this ground of rejection for the reasons discussed below.

Van Voorhis et al. relates to the enrichment and characterization of human dendritic cells from peripheral blood. Van Voorhis et al. does not teach or suggest proliferation of human dendritic cell precursors *in vitro*, nor does it teach or suggest that additional factors that inhibit the maturation or proliferation of non-dendritic cell precursors should be added to cultured medium. Ruley et al. relates to retrovirus promoter-trap vectors and does not even discuss or teach culturing of dendritic cells or any conditions under which proliferating dendritic cells per se should be cultured. Absent such disclosures, neither Van Voorhis et al. or Ruley et al. can render the claimed invention obvious either alone or in combination with Markowicz et al.

As discussed above, Markowicz et al. also does not teach obtaining mature dendritic cells from proliferating dendritic cell precursors or teach that additional factors such IL-4 or IL-13 should be added to the culture to inhibit the maturation or proliferation of non-dendritic cell precursors. Even if *arguendo*, one were to combine Markowicz et al. with Ruley et al. and Van Voorhis et al. there is no teaching regarding culturing proliferating dendritic cells. Absent such disclosures, none of the references can render the claimed invention obvious either alone or in combination. Reconsideration and withdrawal of the 35 U.S.C. § 103 rejection is respectfully requested.

INFORMATION DISCLOSURE STATEMENT

Applicants note that they have not received copies of the initialed PTO-1449 forms submitted in an Information Disclosure Statement filed on August 19, 1996 (via hand delivery to the USPTO). A copy of the PTO-1449 form and date stamped return receipt post

card are attached as Exhibit 3. The IDS were timely filed and applicants therefore respectfully request that the Examiner indicate his review of this IDS by returning of the initialed PTO-1449 form.

AUTHORIZATION

No additional fee is believed due.

The Commissioner is hereby authorized to charge any additional fees which may be required for this Amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2016-4000US3. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

In the event that an extension of time is required or which may be required in addition to that requested in the petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2016-4000US3.

If any questions or issues remain or if the examiner has any comments or suggestions for expediting allowance of this application, he is urged to contact the undersigned at the telephone number below.

Respectfully submitted,

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